

DOCKET NO.: 215869US0PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Nobuya MATSUOKA et al.

SERIAL NO.: NEW U.S. PCT APPLICATION

FILED: HERewith

INTERNATIONAL APPLICATION NO.: PCT/JP00/03334

INTERNATIONAL FILING DATE: May 24, 2000

FOR: AGENT FOR EXPRESSION OF LONG-TERM POTENTIATION OF SYNAPTIC
TRANSMISSION COMPRISING COMPOUND HAVING BRAIN SOMATOSTATIN
ACTIVATION PROPERTY**REQUEST FOR PRIORITY UNDER 35 U.S.C. 120
AND THE INTERNATIONAL CONVENTION**Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In the matter of the above-identified application for patent, notice is hereby given that
the applicant claims as priority:

<u>COUNTRY</u>	<u>APPLICATION NO</u>	<u>DAY/MONTH/YEAR</u>
USA	09/321,745	28 May 1999

Certified copies of the corresponding Convention application(s) were submitted to the
International Bureau in PCT Application No. PCT/JP00/03334. Receipt of the certified
copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been
acknowledged as evidenced by the attached PCT/IB/304.Respectfully submitted,
OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.Norman F. Oblon
Attorney of Record
Registration No. 24,618
Surinder Sachar
Registration No. 34,423

22850

(703) 413-3000
Fax No. (703) 413-2220
(OSMMN 1/97)

28.07.00

JP 00/3334

4

PA 251143

REC'D 11 AUG 2000

WIPO

PCT

THE UNITED STATES OF AMERICA**TO ALL TO WHOM THESE PRESENTS SHALL COME:****UNITED STATES DEPARTMENT OF COMMERCE****United States Patent and Trademark Office****June 14, 2000**

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111.**

APPLICATION NUMBER: 09/321,745**FILING DATE: May 28, 1999****PRIORITY
DOCUMENT**

**SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)**



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

T. Wallace
T. WALLACE
Certifying Officer

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

06/15/1999 VTOWLER 00000017 09321745

01 FC:101	760.00 DP
02 FC:139	130.00 DP

06/23/1999 VTOWLER 00000001 09321745

01 FC:199	708.00 DP
-----------	-----------

PTO-1556

(5/87)

*U.S. GPO: 1998-433-214/80404



#4

SPECIFICATION

AGENT FOR EXPRESSION OF LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION COMPRISING COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY

5

Technical Field

The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, an anti-dementia agent and an anti-amnesia agent, all of which containing a compound having a brain somatostatin activation property. More particularly, the present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, an anti-dementia agent and an anti-amnesia agent, all of which containing a compound exerting a brain somatostatin release promoting action through suppression of the negative feedback mechanism of brain somatostatin release. The present invention moreover relates to a method for expressing long-term potentiation of synaptic transmission, a method for the treatment and/or prophylaxis of dementia and amnesia, and a screening method of these drugs using a somatostatin releasing property as an index.

Background Art

The hippocampal function is said to be responsible for learning and memory. When an input neuron of the hippocampus is stimulated for a short time at high frequency, the efficiency of synaptic transmission continues to increase for a long time thereafter. This phenomenon is called long-term potentiation (hereinafter also referred to as LTP) of synaptic transmission, and has been recognized as a cellular model of learning and memory (T. V. P. Bliss and G. L. Collingridge, Nature vol. 361, p. 31, 1993). There is a demand for further elucidation of the mechanism of the LTP and the relation thereof with learning and memory. Also, a search for a compound having a property of long-term potentiation of synaptic transmission has been desired.

Somatostatin has been known for quite a long time as a hypothalamic hormone capable of suppressing the secretion of somatotropin from the pituitary gland. It has been recently found that it is also present in the cerebral cortex and the hippocampus, that are important cerebral sites for memory and learning, at high concentrations, playing an important role in memory and learning as a

neuromodulator.

Summary of the Invention

As a result of the intensive studies of the present inventors, it has been newly found that a compound having a brain somatostatin release promotion
5 property expresses long-term potentiation of synaptic transmission. Based on this new finding, we have found that administration of a compound having a brain somatostatin release promoting property leads to the prophylaxis and/or treatment of cerebral diseases such as dementia, amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease, and the
10 like, which resulted in the completion of the present invention.

Accordingly, the present invention provides the following.

- (1) An agent for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property as an active ingredient.
- 15 (2) The agent for expression of long-term potentiation of synaptic transmission of (1), wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.
- (3) The agent for expression of long-term potentiation of synaptic transmission of
20 (1) or (2), which is an agent for the prophylaxis or treatment of cerebral diseases.
- (4) The agent for expression of long-term potentiation of synaptic transmission of (3), which is an agent for the prophylaxis or treatment of dementia or amnesia.
- (5) A method for expressing long-term potentiation of synaptic transmission,
25 comprising administering an effective amount of a compound having a brain somatostatin activating property.
- (6) The method for expressing long-term potentiation of synaptic transmission of (5), wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain
30 somatostatin release.
- (7) The method for expressing long-term potentiation of synaptic transmission of (5) or (6), which is a method for the prophylaxis or treatment of cerebral diseases.

- (8) The method for expressing long-term potentiation of synaptic transmission of (5) or (6), which is a method for the prophylaxis and/or treatment of dementia or amnesia.
- 5 (9) Use of a compound having a brain somatostatin activation property for the production of an agent for the expression of long-term potentiation of synaptic transmission.
- 10 (10) The use of a compound having a brain somatostatin activation property according to (9), wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.
- (11) The use of a compound having a brain somatostatin activation property according to (9) or (10), which is for the production of an agent for the prophylaxis and/or treatment of cerebral diseases.
- 15 (12) The use of a compound having a brain somatostatin activation property according to (9) or (10), which is for the production of an agent for the prophylaxis and/or treatment of dementia or amnesia.
- (13) A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises using a somatostatin releasing action as an index.
- 20 (14) The screening method of (13), which is a screening method of an anti-dementia agent or anti-amnesia agent.
- (15) A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises stimulating hippocampal slices, bringing a hippocampal slice into contact with a test compound, measuring an amount of somatostatin released from the hippocampal slice and/or a release time thereof, measuring an amount of somatostatin released from a hippocampal slice and/or a release time thereof in the absence of a contact with the test compound, and comparing the amounts and/or the times to calculate the amount of somatostatin released from the hippocampal slice and/or the release time thereof caused by the contact with the test compound.
- 25 30 (16) The screening method according to (15), which is a screening method of an anti-dementia agent or anti-amnesia agent.
- (17) The agent for expression of long-term potentiation of synaptic

transmission of (1), wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of (13) to (16).

(18) The method for expressing long-term potentiation of synaptic transmission according to (5), wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of (13) to (16).

(19) The use of a compound having a brain somatostatin activation property according to (9), wherein the compound having the brain somatostatin activation property is obtained by the screening method of any of (13) to (16).

(20) A compound selected by the screening method described in any of (13) to (16).

Brief Description of the Drawing

Fig. 1 is a bar graph showing the action of compound 1 to be mentioned later, i.e., N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate, on somatostatin release from rat hippocampal slice upon stimulation with 50mM K⁺, wherein the axis of ordinate is somatostatin release (%) and each value is mean \pm S.E.M (n=10 – 11). The symbol * means that Dunnett's multiple comparison test showed a significant difference by P<0.05 of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 2 is a bar graph showing the action of compound 1 on hippocampus long-term potentiation phenomenon, wherein the axis of ordinate shows the magnitude of LTP by the integral (%·min) of potential variation (%) from 12 minutes to 62 minutes after tetanic stimulation. The symbol * means that one-way analysis of variance and Dunnett's multiple comparison test showed a significant difference by P<0.05 of the group containing various concentrations of compound 1 as compared to the control group. The symbol ** means that one-way analysis of variance and Dunnett's multiple comparison test showed a significant difference by P<0.01 of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 3 is a bar graph showing the dose-response dependency of compound 1 with regard to voltage-dependent calcium channel, wherein the axis of ordinate shows variation (%) of the maximal value of the membrane potential dependent

calcium current to the value before the addition of compound 1, wherein each value is mean \pm S.E.M (n=7). The symbol * means that Dunnett's multiple comparison test showed a significant difference by $P < 0.05$ of the group containing various concentrations of compound 1 as compared to the control group. The symbol ** means that Dunnett's multiple comparison test showed a significant difference by $P < 0.01$ of the group containing various concentrations of compound 1 as compared to the control group.

Fig. 4 is a bar graph showing the action of somatostatin and compound 1 on the membrane potential dependent calcium current, wherein the axis of ordinate shows variation (%) of the maximal value of the membrane potential dependent calcium current upon addition of somatostatin alone or both compound 1 and somatostatin, to the value before addition of somatostatin alone or both compound 1 and somatostatin and each value is mean \pm S.E.M., and the numerals in parentheses are the number of times of measurements. The symbol ** means that Dunnett's multiple comparison test showed a significant difference by $P < 0.01$ of the group containing compound 1 and somatostatin as compared to the group containing somatostatin.

Detailed Description of the Invention

Respective definitions and specific examples thereof used in the present invention, as well as preferable embodiments thereof are explained in detail in the following.

Compound having brain somatostatin activation property:

The activation property of brain somatostatin means, for example, an action to promote release of brain somatostatin, an action to increase biosynthesis of somatostatin within nerve cells, an action to activate somatostatin receptors, an action to potentiate expression of somatostatin property, an action to potentiate somatostatin signal transduction and the like.

The compound to be used in the present invention is one capable of activating brain somatostatin based on at least one of the aforementioned properties. Particularly, a compound having a brain somatostatin release promoting property is preferably used, which is preferably a compound that shows a brain somatostatin release promoting property through suppression of the negative feedback mechanism of brain somatostatin release.

Examples of promotion of release of brain somatostatin include actions of, for example, suppression of a negative feedback mechanism of brain somatostatin release, release of suppression by somatostatin of voltage-dependent calcium channel present in neuron, promotion of the voltage-dependent calcium channel, modification of mutual intracellular action between G protein and calcium channel, phosphorylation of calcium channel, modification of K⁺ channel, influencing kinetic behavior of somatostatin-containing vesicle, and the like, whereby release of somatostatin is promoted.

The action to increase biosynthesis of somatostatin in the nerve cells may be, for example, an action to potentiate the expression of somatostatin mRNA, an action to promote protein synthesis from mRNA, or an action to promote cleaving out from the precursor of somatostatin, wherein these actions promote the release of brain somatostatin.

The brain somatostatin release promoting property is evaluated by the method to be mentioned later.

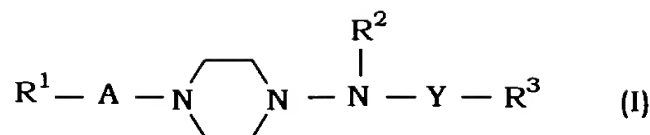
The action to activate somatostatin receptors may be, for example, the actions to stimulate somatostatin receptors, to suppress desensitization of somatostatin receptors, to suppress intracellular transfer of somatostatin receptors, to increase the number of somatostatin receptors present in the postsynaptic membrane and the like.

The action to potentiate the expression of somatostatin property may be, for example, an action to suppress the decomposition of somatostatin, an action to suppress the re-uptake of somatostatin and the like.

The action to potentiate the signal transmission of somatostatin is exemplified by an action to potentiate G protein, cAMP, protein kinase, protein phosphatase, transcription factor and the like, coupled with a somatostatin receptor, wherein the action is directed to an intracellular signal transduction messenger and the like other than somatostatin receptor, thereby to potentiate the signal transduction of somatostatin.

The compound having a brain somatostatin activation property to be used in the present invention encompasses any compound having such an activation property. Preferable examples thereof include compounds of the following formulas:

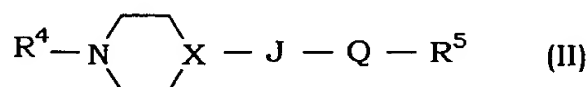
① formula (I)



wherein R^1 is lower alkyl, aryl, ar(lower)alkoxy or heterocyclic group, each of which may be substituted with halogen, R^2 is hydrogen atom or lower alkyl, R^3 is

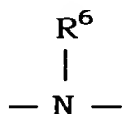
5 cyclo(lower)alkyl, aryl or ar(lower)alkyl, each of which may be substituted with halogen, A is $-\text{CO}-$, $-\text{SO}_2-$ or lower alkylene, and Y is $-\text{CO}-$, $-\text{SO}_2-$ or $-\text{CONH}-$ (EP Publication No. 436734), particularly, N-(4-acetyl-1-piperaziny)-p-fluorobenzamide monohydrate (compound 1) (International Publication No. WO98/25914), and

10 ② formula (II)



wherein R^4 is acyl, R^5 is aryl, arylamino or aryloxy, the aryl moiety of all of which may be substituted by halogen; pyridyl or pyridylamino; X is CH or N, J is a single bond, lower alkylene or

15



wherein R^6 is hydrogen, lower alkyl or N-protective group, Q is $-\text{CH}_2-$, $-\text{CO}-$ or $-\text{SO}_2-$,

provided that when X is N, J is a single bond or lower alkylene,

20 particularly, N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide.

With regard to the brain somatostatin release promoting property, the brain somatostatin release promoting property is said to be expressed and the compound is said to have a brain somatostatin activation property, when the release amount of somatostatin by depolarization stimulation is increased due to a

pretreatment of a hippocampal slice with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the property of increasing biosynthesis of somatostatin, the property of increasing biosynthesis of somatostatin in nerve cells is said to be
5 expressed and the compound is said to have a brain somatostatin activation property, when a somatostatin content of the nerve cells is increased in the nerve cells due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the somatostatin receptor activation property, the
10 somatostatin receptor activation property is said to be expressed and the compound is said to have a brain somatostatin activation property, when binding of somatostatin labeled with a radioisotope (e.g., ^{125}I and the like) to a somatostatin receptor in the nerve cells decreases due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the potentiation property of the expression of somatostatin
15 property, the potentiation property of the expression of somatostatin property is said to be expressed and the compound is said to have a brain somatostatin activation property, when the activity of somatostatin degrading enzyme in the nerve cells is suppressed due to a pretreatment with a certain compound, as
20 compared to when the pretreatment with the compound is void.

With regard to the potentiation property of the somatostatin signal
transmission, the potentiation property of the somatostatin signal transmission is said to be expressed and the compound is said to have a brain somatostatin
activation property, when the amount of intracellular phosphorylated protein in
25 the nerve cells changes due to a pretreatment with a certain compound, as compared to when the pretreatment with the compound is void.

With regard to the property of suppressing the negative feedback
mechanism of brain somatostatin release, the property of suppressing the negative
feedback mechanism of brain somatostatin release is said to be expressed and the
30 compound is said to have a brain somatostatin release promoting property, when the suppressive action on the membrane potential dependent calcium current by somatostatin in the hippocampus pyramidal cells is released due to a pretreatment with a certain compound.

Agent for expression of long-term potentiation of synaptic transmission:

The compound having a brain somatostatin activation property expresses the long-term potentiation of synaptic transmission.

Therefore, this compound is used as an agent for expression of long-term
5 potentiation of synaptic transmission for mammals such as human, dog, cow, horse, rat, guinea pig and the like.

In the present invention, an agent for expression of long-term potentiation of synaptic transmission means a compound capable of inducing potentiation of general neurotransmission efficiency after high frequency and short time
10 stimulation and sustaining the increased transmission efficiency for a long time.

The site where the long-term potentiation of synaptic transmission is expressed is subject to no limitation as long as it is a nerve system present in the hippocampus. Examples thereof include nerve systems present in brain, such as cerebral cortex, corpus amygdaloideum and the like, with preference given to the
15 mossy fiber - CA3 pathway, perforant path-dentate gyrus pathway and Schaffer collaterals-CA1 pathway.

The agent for expression of long-term potentiation of synaptic transmission of the present invention is effective for the prophylaxis and/or treatment of cerebral diseases such as dementia (e.g., senile dementia ,
20 Alzheimer's dementia, dementia associated with various diseases such as cerebral vascular dementia , cerebral post-traumatic dementia, dementia due to brain tumor, dementia due to chronic subdural hematoma, dementia due to normal pressure hydrocephalus, post-meningitis dementia, Parkinson's disease type dementia, and the like), amnesia, manic-depressive psychosis, schizophrenia,
25 Parkinson's disease, psychosomatic disease, and the like, particularly for the treatment and/or treatment of dementia and amnesia.

The long-term potentiation of synaptic transmission is evaluated according to the following criteria.

In a long-term potentiation phenomenon observed in the hippocampus of
30 an animal, namely, guinea pig, which is observed according to the method conventionally known (Matsuoka et al., *Brain Research*, vol. 553, p. 188, 1991), when a pretreatment of hippocampus with a certain compound (bringing hippocampus into contact with the compound) leads to the augmentation of LTP,

after tetanic stimulation, of potential generated from the CA3 field pyramidal cell caused by stimulation of mossy fiber to not less than 120%, preferably not less than 140%, more preferably not less than 160%, still more preferably not less than 180%, most preferably not less than 200%, when that without the pretreatment with the compound is 100%, the long-term potentiation of synaptic transmission is said to be expressed, and this compound can be said to be an agent for expression of long-term potentiation of synaptic transmission.

Alternatively, in a long-term potentiating phenomenon observed in the hippocampus of an animal, namely, guinea pig, which is observed according to the method conventionally known (Matsuoka et al., *Brain Research*, vol. 553, p. 188, 1991), when a long-term potentiation phenomenon is expressed in a sustained manner for not less than 10 minutes, preferably not less than 20 minutes, more preferably not less than 30 minutes, most preferably not less than 60 minutes due to a pretreatment with a certain compound, a long-term potentiation of synaptic transmission is said to be expressed and this compound can be said to be an agent for expression of long-term potentiation of synaptic transmission.

Note that variations in numerical values to the degree that those of ordinary skill in the art consider substantially the same statistically as the above-mentioned numerical values should be construed as corresponding to the above-mentioned numerical values.

As used herein, the pretreatment with a certain compound can be conducted by, for example, immersing the compound generally for 25 minutes in an outer solution containing hippocampus therein, and removing the compound by a method such as washing.

The test method for the evaluation of the above-mentioned long-term potentiation of synaptic transmission may be a method conventionally known or a method analogous thereto, with preference given to the following method.

Round slices are prepared from the hippocampus removed from an animal, for example, guinea pig. The slices are 500 μm in thickness. Each slice is placed in a container such as a perfusion chamber and the population spikes are extracellularly recorded in the following manner while perfusing with an artificial cerebrospinal fluid at 33 - 34°C at a flow rate of 1.8 - 2.0 ml/min.

At this time, the test compound is added to the perfusate so that the

concentration of the test compound in each container would be a serially diluted concentration, and the test compound is applied to the hippocampal slices from 21 minutes before the tetanic stimulation to 4 minutes thereafter.

5 The artificial cerebrospinal fluid to be used here is exemplified by those used in the screening method in the present invention to be mentioned later.

Mossy fiber is stimulated with a stimulation electrode at a voltage of not more than 10 V, frequency of 0.2 Hz, and the population spikes in the CA3 field pyramidal cell layer are recorded every 5 minutes. When the population spikes to be recorded are stabilized, tetanic stimulation is applied for the induction of LTP.

10 The tetanic stimulation includes stimulation at the same voltage and a frequency of 33 Hz for 5 seconds. The height of from the negative peak to the next positive peak of the obtained population spikes after stimulation is measured and taken as an amplitude of population spikes. The degree of LTP can be expressed by a potential variation (%) showing an increase in the amplitude of population spikes

15 after tetanic stimulation relative to the average of 4 measurements of the amplitudes of population spikes before tetanic stimulation. As the index of the property of a drug, the area in the graph of time lapse versus potential variation, between the graph from 12 minutes to 62 minutes after tetanic stimulation and the line where the potential variation is 0%, may be calculated and taken as the

20 magnitude of LTP.

Each value of the potential variation and the magnitude of LTP which is calculated based on the potential variation as mentioned above is subjected to one-way analysis of variance and Dunnett's multiple comparison test, and compared based on the above-mentioned evaluation criteria, whereby the long-

25 term potentiation of synaptic transmission can be evaluated.

screening method

The screening method of the present invention comprises screening of the test compound to be screened (hereinafter to be abbreviated as a test compound) using the somatostatin releasing property as an index.

30 The screening method of the agent for expression of long-term potentiation of synaptic transmission of the present invention, particularly, an anti-dementia agent and an anti-amnesia agent, is a selection method of the test compound characteristically comprising bringing human or animal nerve cells into contact

with a test compound, measuring an amount of somatostatin released from the nerve cells and a release time thereof, measuring an amount of somatostatin released from the nerve cells and a release time thereof in the absence of a contact with the test compound, and comparing the amounts and the times to measure the amount of somatostatin released from the nerve cells and the release time thereof caused by the contact with the test compound.

The above-mentioned nerve cells include, for example, hippocampus, preferably hippocampal slices, primary culture nerve cell, nerve cell strain, oocyte and the like.

The contact with the test compound can be conducted by, for example, immersing the test compound for 20 - 120 minutes in an outer solution in which the nerve cells are immersed and removing the test compound by washing and the like.

Preferred screening method is as follows.

Hippocampal slices are prepared from an animal (e.g., rat, guinea pig, mouse and the like) by a method conventionally known or a method analogous thereto. The slices are generally 100 - 600 μm , preferably 300 - 400 μm , in thickness. While the direction of slicing is not particularly limited, they are preferably transverse slices or longitudinal slices. For example, the slices are placed in a container such as a perfusion chamber, by generally 30 slices, more preferably 20 slices, and perfused with an artificial cerebrospinal fluid while incubating at generally 20 - 37°C, preferably 36 - 37°C. The perfusate is exchanged every 5 - 20 minutes, preferably 10 - 15 minutes. Fractions are obtained from each cycle, which step is repeated 10 to 20 times, preferably 15 times. While repeatedly obtaining the fractions, the test compound is added to the perfusate to serially diluted concentrations that are within the range of preferably 10^{-10} - 10^{-5} M, more preferably 10^{-9} - 10^{-6} M, whereby fractions containing the test compound at respective concentrations are obtained. The test compound is immersed in perfusate for generally 20 - 120 minutes, preferably 80 minutes.

Generally 0 - 60 minutes, preferably 10 - 30 minutes, after the addition of the test compound, stimulation is preferably added. The stimulation is applied for generally 5 - 20 minutes, preferably 10 - 15 minutes. It is important that the

stimulation be applied after the addition of the test compound, but the stimulation may be applied while the test compound is present in the perfusate or after the test compound is removed by washing with a perfusate.

5 The somatostatin in the perfusate of each fraction obtained as above is quantified according to a treatment method conventionally known (for example, perfusate is lyophilized and subjected to a radioimmunoassay). After the completion of the step for obtaining the fractions, the somatostatin remaining in hippocampal slices is extracted by a conventional method, which amount is similarly quantified.

10 The composition of the artificial cerebrospinal fluid to be used as the perfusate in the above-mentioned screening method can be modified as appropriate depending on the test conditions and test compound and the like to be used. Preferable composition includes the following.

15 Artificial cerebrospinal fluid composition: NaCl, 124 mM; KCl, 5 mM; KH_2PO_4 , 1.24 mM; MgSO_4 , 1.3 mM; CaCl_2 , 2.4 mM; NaHCO_3 , 26 mM; D-glucose, 10 mM. This perfusate is used with saturation with a mixed gas of oxygen and carbon dioxide, such as a mixed gas of oxygen (95%) and carbon dioxide (5%), typically used for pharmacological experiments.

 The above-mentioned screening method may be modified as necessary.

20 The amount of somatostatin quantified is compared with the amount when the contact with the test compound is void, based on the following criteria, whereby the somatostatin release promoting property of the test compound can be evaluated.

25 The screening method of the present invention is preferably conducted under stimulation as mentioned above. Such stimulation is a model of a specific stimulation related to learning or tetanic stimulation. The stimulation is not particularly limited as long as the nerve cells present in the hippocampal slice are excited. Specific examples thereof include stimulation by potassium ion, electric stimulation, depolarization stimulation, stimulation with a drug and the like.

30 When a mere addition of the test compound does not lead to the somatostatin release property and the test compound shows somatostatin release property only upon stimulation, it can be a confirmation that the nerve cells are free from influence of this test compound as long as no stimulation is involved, thus

ensuring the safety of this compound.

The somatostatin release property is evaluated based on the following criteria.

In the screening method of the present invention, when the somatostatin
5 release amount due to the contact with the test compound increases by not less
than 10%, preferably not less than 20%, more preferably not less than 30%, still
more preferably not less than 40%, most preferably not less than 50%, as
compared to the release amount when the contact with the test compound is void
(which is taken as 100%), the test compound is said to have a somatostatin release
10 promoting property. It is preferable that the somatostatin release amount upon
stimulation mentioned above be evaluated according to the above-mentioned
criteria. It is appreciated that variations in numerical values to the degree that
those of ordinary skill in the art consider substantially the same statistically as the
above-mentioned numerical values should be construed as corresponding to the
15 above-mentioned numerical values

The test compound to be subject to the screening in the present invention
is free of any particular limitation and may be selected from natural product,
chemically synthesized compound, nucleic acid, peptide, antibody and the like
obtained by genetic engineering and their libraries. The test compound is
20 preferably a pure substance, but may be a mixture or racemic compound. The
test compound may be also modified to label with radioisotope or may contain
modification made during construction of library. The obtained test compound
can be optimized by chemical synthetic method and the like.

By selecting the test compounds using the screening method of the
25 present invention, an agent for expression of long-term potentiating of synaptic
transmission, particularly an anti-dementia agent, an anti-amnesia agent and the
like can be screened.

The test compound selected by the screening method of the present
invention and a compound obtained by optimizing this compound are all
30 encompassed in the scope of the present invention.

The compound of the present invention having a brain somatostatin
activation property can be used in the dosage form of a solid, semi-solid or liquid
preparation in conjunction with organic or inorganic carrier or excipient, which is

suitable for rectal administration, pulmonary (pernasal or buckle inhalation), nasal drop, eye drop, external (local), oral or parenteral (subcutaneous, intravenous or intramuscular) administration and the like, direct administration to diseased region, such as brain, spinal fluid, cerebroventricle and the like, or
5 inhalation.

A compound having a brain somatostatin activation property can be admixed with pharmaceutically acceptable substantially non-toxic carrier or excipient conventionally used for dosage forms suitable for use, such as tablets, pellets, troches, capsules, suppositories, cream, ointment, aerosol, inhalable
10 powder medicine, liquid, emulsion, suspension, and the like. Where necessary, auxiliary, stabilizer, tackifier, coloring agent and flavor can be used.

The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia agent, of the present invention can be produced by a method conventionally used in the
15 pertinent field. Where necessary, a method routinely used in this technical field can be used for the production of these drugs for an improved bioavailability.

The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia agent, of the present invention is preferably administered intravenously (inclusive of
20 addition into infusion), intramuscularly or orally when applying to humans or animals.

The agent for the expression of long-term potentiation of synaptic transmission, particularly an anti-dementia agent and an anti-amnesia agent, of the present invention is contained in a preparation in an amount sufficient to
25 provide a desired prophylactic and/or treatment effect on the progression and conditions of diseases.

The amount and administration route of the compound having a brain somatostatin activation property are subject to variation depending on the kind of compound, age and conditions of the patients to be the subject of the prophylaxis
30 and/or treatment. When compound 1 is used, for example, the daily dose is 0.1 - 10 mg/kg body weight by oral administration, which is given once to several times a day for the treatment and/or prophylaxis of the aforementioned diseases.

The present invention is explained in more detail in the following by way of

Examples which do not limit the present invention.

Examples

Experimental Example 1: Effect of compound 1 on somatostatin release from rat hippocampal slice

5 (1) method

Rat hippocampal slices (thickness 350 μ m, round slice) were prepared by a standard method. Twenty rat hippocampal slices were placed in a perfusion chamber, incubated at 37°C and perfused by a batch method while exchanging the incubation buffer every 10 minutes. The incubation buffer used had the
10 composition as noted below. A mixed gas of oxygen (95%) and carbon dioxide (5%) was used to saturate the buffer.

Composition of incubation buffer: NaCl, 124 mM; KCl, 5 mM; KH_2PO_4 , 1.24 mM; MgSO_4 , 1.3 mM; CaCl_2 , 2.4 mM; NaHCO_3 , 26 mM; D-glucose, 10 mM

Perfusion for 150 minutes gave fractions 1-15. To fraction 9 was applied
15 a high K^+ (50 mM) stimulation. Compound 1 was added to fractions 7 - 15 to the concentration of 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, respectively. Nothing was added to control group. The respective fractions thus obtained were concentrated by lyophilization and somatostatin in the perfusate was quantified by
20 radioimmunoassay (RIA). After the completion of the experiment, somatostatin remaining in the slices was extracted by a conventional method and quantified by radioimmunoassay. The somatostatin amount released by high K^+ (50 mM) stimulation was calculated and the amount of somatostatin released due to the property of compound 1 was measured.

Somatostatin release (%) by compound 1 at each concentration was
25 calculated as in the following. The somatostatin amount of each fraction was expressed by the percentage (%) relative to the somatostatin residual amount at the time the fraction was obtained. The value of fraction 8 immediately before high K^+ (50 mM) stimulation was taken as the base and the values exceeding the base value were added with regard to fraction 9 and the subsequent peak fractions
30 exceeding the base value to give somatostatin release (%). The number of the test samples measured was 10 or 11. Each value (%) was expressed by mean \pm S.E.M. The property of compound 1 was subjected to Dunnett's multiple comparison test relative to control group.

(2) Result

The results are shown in Fig. 1. The compound 1 promoted somatostatin release when stimulated at high K⁺ (50 mM), and the property was significant at 10⁻⁷ M and 10⁻⁶ M. The concentration dependency was similar to the long-term potentiation in Experimental Example 2 to be mentioned later. By the use of the screening method of the present invention, compound 1 was shown to have a somatostatin release promoting property on hippocampus.

Experimental Example 2: Effect of compound 1 on LTP in mossy fiber - CA3 field pyramidal cell of hippocampal slice

(1) method

Slices (thickness 500 μ m, round slice) were prepared from hippocampus removed from male Hartley guinea pigs (body weight 220 - 350 g) and population spikes were extracellularly recorded. The hippocampal slices were perfused with an artificial cerebrospinal fluid (33-34°C, composition: NaCl, 124 mM; KCl, 5 mM; KH₂PO₄, 1.24 mM; MgSO₄, 1.3 mM; CaCl₂, 2.4 mM; NaHCO₃, 26 mM; D-glucose, 10 mM) saturated with a mixed gas of oxygen (95%) and carbon dioxide (5%) at flow rate of 1.8 - 2.0 ml/min. Mossy fiber was stimulated with a stimulating electrode at a voltage of not more than 10 V, frequency of 0.2 Hz and the population spikes in the CA3 field pyramidal cell layer was recorded every 5 minutes. When the population spikes to be recorded were stabilized, tetanic stimulation for induction of LTP was applied. The tetanic stimulation includes stimulation at the same voltage of not more than 10 V, as when the stimulation was applied at a frequency of 0.2 Hz, but upon increase of the frequency to 33 Hz for 5 seconds. The height of from negative peak to the next positive peak of the obtained population spikes after stimulation was measured and taken as an amplitude (population spike amplitude; PSA). The degree of LTP was expressed by a potential variation (%) by the increase in the amplitude of population spikes after tetanic stimulation relative to the average of 4 measurements of the amplitudes of population spikes obtained before tetanic stimulation. Each value was expressed by mean \pm S.E.M (the number of slices tested was 3 to 8). As the index of the property of a drug, the area (% \cdot min) between the graph from 12 minutes to 62 minutes after tetanic stimulation and the line where the potential variation is 0%, in the time lapse graph of potential variation, was calculated and

taken as the magnitude of LTP. Compound 1 was dissolved in and diluted with distilled water and added to perfusate to the concentration of 10^{-9} M, 10^{-8} M, 10^{-7} M and 10^{-6} M, respectively, and applied to a hippocampal slice for 25 minutes from 2 minutes before the tetanic stimulation to 4 minutes thereafter. Nothing was added to perfusate for control group.

The property of compound 1 relative to the magnitude of LTP was tested by one-way analysis of variance and Dunnett's multiple comparison test.

(2) Results

From the potential variation with the lapse of time, compound 1 was found to have shown no significant influence of the response of base before tetanic stimulation. As is can be seen from Fig. 2 showing the magnitude of LTP, compound 1 increased potential variation after tetanic stimulation at 10^{-7} M and 10^{-6} M, wherein its action was maximal at 10^{-7} M. From the foregoing, it was shown that compound 1 had a promoting property specific to the mechanism necessary for the occurrence of phenomenon of long-term potentiation of synaptic transmission after tetanic stimulation.

The hippocampal slice obtained from the guinea pig treated with cysteamine (200 mg/kg) did not show LTP enhancing property by compound 1 at 10^{-7} M. This reveals that hippocampus endogenous somatostatin activation property is involved in the LTP enhancing property by compound 1.

Experimental Example 3: Effect of compound 1 on voltage-dependent Ca^{2+} current of rat hippocampus pyramidal cell

(1) method

Hippocampal slices were prepared from the brains of 5- to 14-day-old male Wistar rats and the nerve cells were quickly isolated by trypsin enzyme treatment method. According to the whole cell patch-clamp method, the membrane potential of hippocampus pyramidal cell was fixed at -80-mV and the membrane potential-dependent potassium current (I_{K}) was measured in Cs^+ electrode inner solution and Ba^{2+} bath outer solution. The resistance of the electrode was $2 \sim 4 \text{ M}\Omega$. Compound 1 was added to bath outer solution concentrations of $0.01 \mu\text{M}$, $0.1 \mu\text{M}$ and $1.0 \mu\text{M}$, respectively, and perfused with the bath outer solution. Nothing was added to the bath outer solution for the control group. The maximal value of the current (peak amplitude) was measured, and

calculated as the variation (%) relative to the value before addition of compound 1. Each value (%) was expressed by mean \pm S.E.M. The number of measurements was 7. The property of compound 1 was evaluated by Dunnett's multiple comparison test relative to the control group.

5 (2) Result

The results are shown in Fig. 3. Compound 1 significantly promoted membrane potential-dependent calcium current at concentrations of 0.01 μ M, 0.1 μ M and 1.0 μ M as compared to the control group, and showed bell-shape dose dependency. Therefore, compound 1 was shown to have a promoting action on the voltage-dependent calcium channel of hippocampus pyramidal cells.

Experimental Example 4: Effect of somatostatin and compound 1 on voltage-dependent Ca^{2+} current of rat hippocampus pyramidal cell

(1) method

In the same manner as in Experimental Example 3 except that compound 1 and somatostatin were added in such a manner that the concentration of somatostatin in the bath outer solution was 10^{-7} M when somatostatin alone was added, and the concentration of compound 1 in the bath outer solution was 10^{-7} M and the concentration of somatostatin in the bath outer solution was 10^{-7} M when both compound 1 and somatostatin were added, the experiment was conducted. Nothing was added to the control group. The maximal value of each current was measured, and calculated as the variation (%) to that prior to addition. Each value (%) was expressed by mean \pm S.E.M. The number of measurements of the group added with somatostatin was 11 and that of the group added with both compound 1 and somatostatin was 7. The both groups were subjected to Dunnett's multiple comparison test.

(2) Result

The results are shown in Fig. 4. While somatostatin evidently suppressed membrane potential-dependent calcium current, suppressive property of somatostatin was completely released in the presence of compound 1. Hence, compound 1 was shown to release the suppressive property of somatostatin on the voltage-dependent calcium channel of hippocampus pyramidal cell and has a promoting property. From the results of Experimental Example 3 and this Experimental Example, compound 1 was shown to suppress the negative feedback mechanism of brain somatostatin release.

WHAT IS CLAIMED IS

1. An agent for expression of long-term potentiation of synaptic transmission, which comprises a compound having a brain somatostatin activation property as an active ingredient.

5

2. The agent for expression of long-term potentiation of synaptic transmission of claim 1, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

10

3. The agent for expression of long-term potentiation of synaptic transmission of claim 1 or 2, which is an agent for the prophylaxis or treatment of cerebral diseases.

15

4. The agent for expression of long-term potentiation of synaptic transmission of claim 3, which is an agent for the prophylaxis or treatment of dementia or amnesia.

20

5. A method for expressing long-term potentiation of synaptic transmission, comprising administering an effective-amount of a compound having a brain somatostatin activation property.

25

6. The method for expressing long-term potentiation of synaptic transmission of claim 5, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

30

7. The method for expressing long-term potentiation of synaptic transmission of claim 5 or 6, which is a method for the prophylaxis or treatment of cerebral diseases.

8. The method for expressing long-term potentiation of synaptic transmission of claim 5 or 6, which is a method for the prophylaxis and/or treatment of

dementia or amnesia.

9. Use of a compound having a brain somatostatin activation property for the production of an agent for the expression of long-term potentiation of synaptic transmission.

5

10. The use of a compound having a brain somatostatin activation property according to claim 9, wherein the compound exerts an action to promote a release of brain somatostatin through suppression of a negative feedback mechanism of brain somatostatin release.

10

11. The use of a compound having a brain somatostatin activation property according to claim 9 or 10, which is for the production of an agent for the prophylaxis and/or treatment of cerebral diseases.

15

12. The use of a compound having a brain somatostatin activation property according to claim 9 or 10, which is for the production of an agent for the prophylaxis and/or treatment of dementia or amnesia.

13. A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises using a somatostatin releasing action as an index.

20

14. The screening method of claim 13, which is a screening method of an anti-dementia agent or anti-amnesia agent.

25

15. A method for screening an agent for expression of long-term potentiation of synaptic transmission, which comprises stimulating hippocampal slices, bringing a hippocampal slice into contact with a test compound, measuring an amount of somatostatin released from the hippocampal slice and/or a release time thereof, measuring an amount of somatostatin released from a hippocampal slice and/or a release time thereof in the absence of a contact with the test compound, and comparing the amounts and/or the times to

30

calculate the amount of somatostatin released from the hippocampal slice and/or the release time thereof caused by the contact with the test compound.

5 16. The screening method according to claim 15, which is a screening method of an anti-dementia agent or anti-amnesia agent.

10 17. The agent for expression of long-term potentiation of synaptic transmission of claim 1, wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of claim 13 to claim 16.

15 18. The method for expressing long-term potentiation of synaptic transmission according to claim 5, wherein the compound having the brain somatostatin activation property is a compound obtained by the screening method of any of claim 13 to claim 16.

20 19. The use of a compound having a brain somatostatin activation property according to claim 9, wherein the compound having the brain somatostatin activation property is obtained by the screening method of any of claim 13 to claim 16.

20. A compound selected by the screening method described in any of claim 13 to claim 16.

ABSTRACT OF THE DISCLOSURE

The present invention relates to an agent for the expression of long-term potentiation of synaptic transmission, which contains a compound having a brain somatostatin activation property as an active ingredient and to a screening
5 method of an agent for the expression of long-term potentiation of synaptic transmission, which uses a somatostatin releasing property as an index. The present invention is useful for the prophylaxis and/or treatment of cerebral diseases of dementia, amnesia, manic-depressive psychosis, schizophrenia, Parkinson's disease, psychosomatic disease and the like.

10

Declaration, Power of Attorney and Petition

Page 1 of 3

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

AGENT FOR EXPRESSION OF LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION

COMPRISING COMPOUND HAVING BRAIN SOMATOSTATIN ACTIVATION PROPERTY

the specification of which

- ☐ is attached hereto.
- ☒ was filed on May 28, 1999 as
Application Serial No. 09/321,745
and amended on _____
- ☐ was filed as PCT international application
Number _____
on _____
and was amended under PCT Article 19
on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b,) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

_____ (Application Number)	_____ (Filing Date)
_____ (Application Number)	_____ (Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)
_____	_____	_____
_____	_____	_____
_____	_____	_____

And we (I) hereby appoint: Norman F. Oblon, Registration Number 24,618; Marvin J. Spivak, Registration Number 24,913; C. Irvin McClelland, Registration Number 21,124; Gregory J. Maier, Registration Number 25,599; Arthur I. Neustadt, Registration Number 24,854; Richard D. Kelly, Registration Number 27,757; James D. Hamilton, Registration Number 28,421; Eckhard H. Kuesters, Registration Number 28,870; Robert T. Pous, Registration Number 29,099; Charles L. Gholz, Registration Number 26,395; Vincent J. Sunderdick, Registration Number 29,004; William E. Beaumont, Registration Number 30,996; Steven B. Kelber, Registration Number 30,073; Robert F. Gnuse, Registration Number 27,295; Jean-Paul Lavalleye, Registration Number 31,451; Timothy R. Schwart, Registration Number 32,171; Stephen G. Baxter, Registration Number 32,884; Martin M. Zoltick, Registration Number 35,745; Robert W. Hahl, Registration Number 33,893; Richard L. Treanor, Registration Number 36,379; Steven P. Weihrouch, Registration Number 32,829; John T. Goolkasian, Registration Number 26,142; Marc R. Labgold, Registration Number 34,651; William J. Healey, Registration Number 36,160; Richard L. Chinn, Registration Number 34,305; Steven E. Lipman, Registration Number 30,011; Carl E. Schlier, Registration Number 34,426; James J. Kulbaski, Registration Number 34,648; Catherine B. Richardson, Registration Number 39,007; Richard A. Neifeld, Registration Number 35,299; J. Derek Mason, Registration Number 35,270; and Jacques M. Dulin, Registration Number 24,067; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose Post Office Address is: Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Nobuya Matsuoka
NAME OF FIRST SOLE INVENTOR

Residence: Ikoma-shi, Nara, Japan

Nobuya Matsuoka
Signature of Inventor

Citizen of: Japan

Post Office Address: 25-9, Kitayamato 2-chome, Ikoma-shi,
Nara 630-0121 Japan

June 22, 1999
Date

Masamichi Satoh
NAME OF SECOND JOINT INVENTOR

Masamichi Satoh
Signature of Inventor

June 23, 1999
Date

NAME OF THIRD JOINT INVENTOR

Signature of Inventor

Date

NAME OF FOURTH JOINT INVENTOR

Signature of Inventor

Date

NAME OF FIFTH JOINT INVENTOR

Signature of Inventor

Date

Residence: Kyoto-shi, Kyoto, Japan

Citizen of: Japan

Post Office Address: 23-3, Shimogamo-Yakocho, Sakyo-ku,
Kyoto-shi, Kyoto 606-0837 Japan

Residence: _____

Citizen of: _____

Post Office Address: _____

Residence: _____

Citizen of: _____

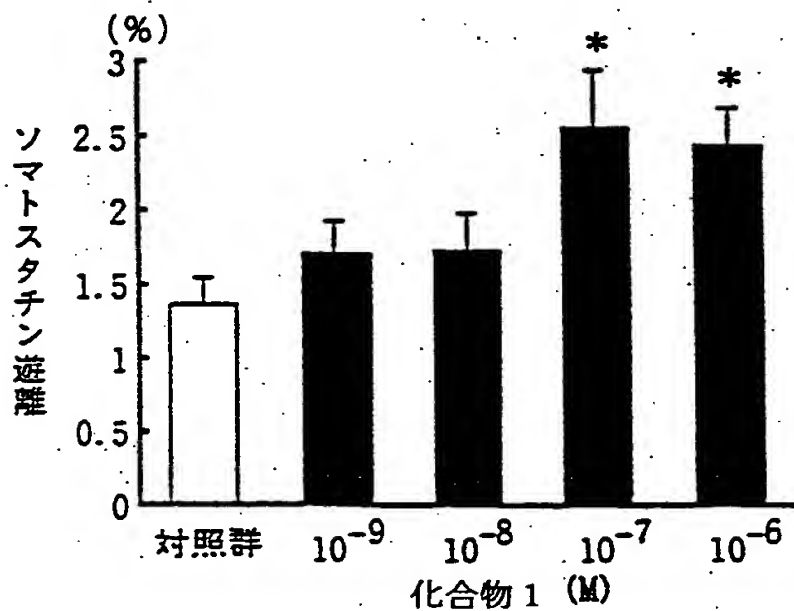
Post Office Address: _____

Residence: _____

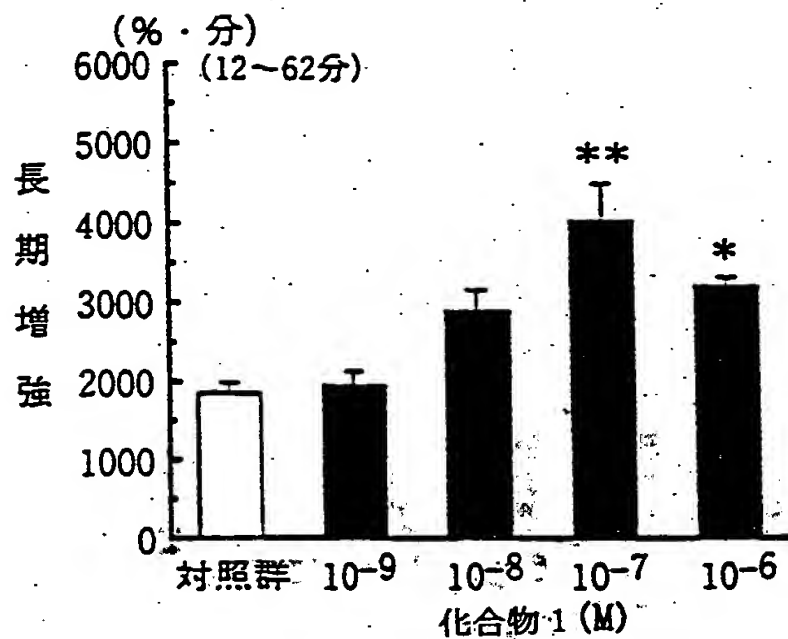
Citizen of: _____

Post Office Address: _____

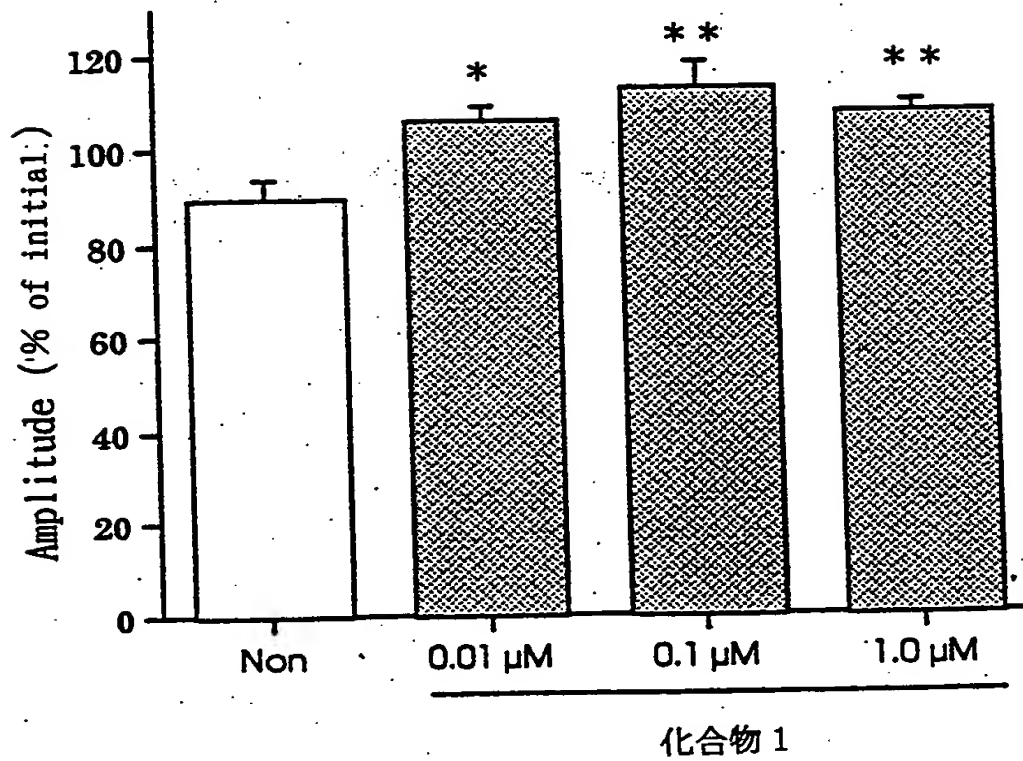
1



2



3



4

